Comparison of ZORBAX Poroshell 300Extend-C18 and Totally Porous Packing in Achieving Very Rapid, High-pH Separation of Peptides

Application

Biotechnology/QA/QC/Basic R&D

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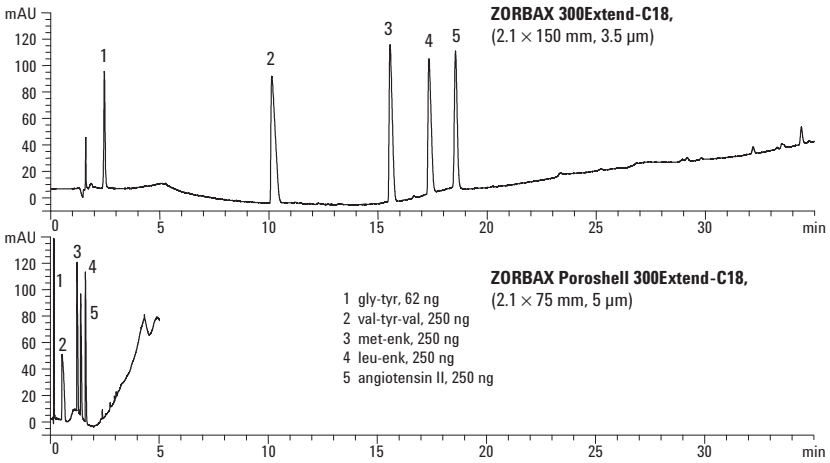
It is often desirable to reduce the time of an HPLC separation. The time saved is beneficial in basic research, as well as pharmaceutical research, product development, and product QA and QC. The superficially porous particles of ZORBAX Poroshell packings facilitate extremely rapid HPLC separations of large molecules such as proteins. These fast separations result from the very rapid rate at which biomolecules can move into and out of the thin superficially porous layer. The separation of peptides and small proteins at high pH is now possible through the use of Extend-C18-bonded Poroshell particles. The high pH may be desirable for improving selectivity, solubility, ease of downstream processing, or to change signal-to-noise and charge-state in LC/MS. The chromatograms below demonstrate the extreme savings in run time achieved using Poroshell 300Extend-C18 over a comparable, totally porous material. Alternatively, gradient times may be extended to achieve even greater resolution in typical run times of 10 to 20 min.

Highlights

- ZORBAX Poroshell columns permit up to five times faster separation of peptides/proteins over columns using totally porous silica packing.
- ZORBAX Poroshell 300Extend-C18 columns permit use of high pH separations to improve selectivity, solubility, and downstream processing.



Mobile phase

A: 10 mM NH_4OH, H_2O

B: 10 mM NH_4OH, MeOH

Gradient timetable

Upper:

0% to 100% B in 40 min

Flow: 0.2 mL/min

Lower:

0% to 100% B in 4 min

Flow: 1.0 mL/min

Conditions

Column	**ZORBAX 300Extend-C18** (2.1 × 150 mm, 3.5 µm) (p/n 763750-902) **ZORBAX Poroshell 300Extend-C18** (2.1 × 75 mm, 5 µm) (p/n 670750-902)
Sample	Peptide standards
Detection	UV (215 nm)
Temperature	Ambient
Injection	0.25 µL

The chromatograms were obtained using a totally porous ZORBAX 300Extend-C18, 3.5-µm packing and a superficially porous ZORBAX Poroshell 300Extend-C18, 5-µm packing. Both packings were used in 2.1-mm diameter columns; however, the totally porous material was used in a column 150 mm in length because of the common use of this size for peptide mapping. Poroshell was used in the standard 75-mm length. A typical 0.2 mL/min was used for the totally porous packing, while the Poroshell column was run at its optimum flow rate of 1 mL/min. While this is not a direct comparison, it demonstrates the common conditions for use of these two different technologies in peptide analyses. Since the structure of Poroshell packing allows Poroshell 300Extend-C18 to be run at a flow rate five times that for a standard packing, and the column is half as long, a ten-fold reduction in run time is demonstrated for Poroshell. Separation of the five peptides takes 40 min on the totally porous material and only 4 min on Poroshell, as seen in the chromatograms. Not only is the separation faster, but the re-equilibration time is also reduced five-fold, so that turn-around times are very rapid. As a result of its speed, Poroshell columns are very useful for QA, QC, basic research, and method development, as method conditions can be rapidly changed and re-assessed. ZORBAX Poroshell Extend is particularly useful because of its stability of operation at both very low and very high pH. (See Agilent publication 5989-0676EN [1].)

Reference

1. Robert Ricker, "Using ZORBAX Poroshell 300Extend-C18 to Achieve Unique Selectivity at pH 2 and 10: Angiotensins," Agilent Technologies, publication 5989-0676EN www.agilent.com/chem

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.
Search "Poroshell".

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